# *Xylella fastidiosa* Diversity

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## ABSTRACT

The bacterial plant pathogen *Xylella fastidiosa* causes disease in a wide range of host plant species. However, the species is very diverse phylogenetically and phenotypically. There are four broadly recognized subspecies of *X. fastidiosa*, which primarily cause disease in a host specific manner. Furthermore, clusters within subspecies may also be host specific. Despite the fact that host specificity is an important characteristic of *X. fastidiosa* phylogenetic clusters, the determinants of specificity are unknown; this bacterium lacks a type III secretion system and effectors, often associated with specificity in other bacterial plant pathogens. Furthermore, field populations have been shown to have large genetic diversity. Because horizontal gene transfer, rearrangements, and other mutational processes appear to be frequent in *X. fastidiosa*, understanding how it evolves is of importance, especially in face of the fact that new diseases caused by this bacterium continue to emerge.

**Keywords**: *Xylella fastidiosa*, vector-borne, Pierce's disease, Pear leaf scorch, xylem.

## INTRODUCTION

The first disease caused by the bacterium *Xylella fastidiosa*, Pierce's disease of grapevines, was described in 1892 by Newton Pierce. An epidemic in California, USA, in the 1930-40s led to a large number of advancements in our knowledge of this and other *X. fastidiosa* diseases, including the identification of insect vectors [28, 29]. Nevertheless, only in the 1970s was the bacterial etiology of these diseases demonstrated [11], as prior to that they were thought to be caused by viruses. This breakthrough resulted in the *in vitro* cultivation of the bacterium [9], which was a decade later named *Xylella fastidiosa* [38]. More recently, it became the first bacterial plant pathogen to be fully sequenced [32], and epidemics in citrus and grapevines in

Brazil and California, USA, respectively, led to an increase in interest and research on this bacterium. The history of important phases of *X. fastidiosa* research has been elegantly summarized in a recent review [25].

There are several reviews that have addressed various aspects of the biology and ecology of *X. fastidiosa* [2, 4, 12, 25, 27]. The specific goal of this article is to summarize current information available on the diversity of *X. fastidiosa*. Furthermore, because it has been shown that this bacterium has a dynamic genome, and that recombination among subspecies occurs in the field, a robust knowledge about its evolution may assist in the development of strategies to reduce the impact of emerging diseases. This is relevant because, in the last decade, several new *X. fastidiosa* diseases have been described in North and Central America, in addition to Taiwan [34].

## The species *Xylella fastidiosa*

*Xylella fastidiosa* is a gamma-proteobacterium in the family Xanthomonadaceae [38]. The main sister clade to *Xylella* is the genus *Xanthomonas* [23]. There are important and significant differences among these bacteria; for example, *Xanthomonas* spp. do not require insect vectors for dispersal, while *X. fastidiosa* does. In addition, the genome of *X. fastidiosa* is much smaller than that of *Xanthomonas* spp., which may be a consequence of a lifestyle fully dependent of plant and insect hosts [23]. However, there are several similarities between these two genera at the genome level, resulting in substantial degree of gene conservation. For example, both produce exopolysaccharides via a highly conserved operon [8, 15], and cell-cell signaling is mediated by another conserved set of genes [17]. A comparison between *Xanthomonas* spp. and *X. fastidiosa* is available elsewhere [4, 23].

Although generally thought of as a plant pathogen, *X. fastidiosa* needs to colonize both its plant and insect hosts. Plant colonization is a process that has received more attention, and is therefore better understood from both biological and mechanistic perspectives (see 4 for a review). Bacterial colonization of plants is based on multiplication within xylem vessels and movement through the xylem network via pit membranes. Vectors of *X. fastidiosa* are xylem sap-sucking insects such as sharpshooter leafhoppers (Hemiptera, Cicadellidae) and spittlebugs (Hemiptera, Cercopidae) [2]. Insect colonization is more poorly studied, but the current hypothesis proposes that *X. fastidiosa* forms a biofilm on the cuticular surface of the foregut of vectors, much like other biofilm-forming bacteria [13]. In addition, there are two other

essential steps to the biology of *X. fastidiosa*. First, the bacterium must be able to initiate an insect colonization event when acquired from an infected plant, a process that is mediated by cell-surface adhesins [14]. Second, once inhabiting a vector it must be dislodged from the cuticle and be inoculated into a susceptible host plant. The processes involved in this step are yet to be determined.

## *Xylella fastidiosa* subspecies

The species *X. fastidiosa* has a very broad host range, one study estimated that species in 29 plant families are susceptible to colonization [10]. However, colonization does not equal disease development. In fact, most plant species that are capable of sustaining a *X. fastidiosa* infection are asymptomatic hosts [24]. In addition, *X. fastidiosa* phylogenetic clusters are largely host specific, meaning they cause disease in one or few host plants, while being able to colonize many asymptomatically [e.g. 1]. Therefore, although at the species level this bacterium has a very large number of hosts, at finer levels of phylogenetic resolution its range is very limited. It should be mentioned that, up to now, even the most divergent *X. fastidiosa* isolates share more than 97% 16S rRNA gene homology, suggesting that they belong to the same bacterial species.

The species is subdivided into four subspecies based on DNA:DNA hybridization [31] and multi-locus sequence typing [MLST, 30]. The subspecies are *fastidiosa*, *multiplex*, *pauca*, and *sandyi*. A fifth subspecies has been proposed [26], but its placement needs to be accurately determined with a larger number of loci or other approaches [21]. Lastly, the cluster that causes pear leaf scorch in Taiwan, representing the most divergent *X. fastidiosa* genotype known so far, will eventually be classified as another subspecies [33]. The subdivision at the subspecies level is not only of taxonomical importance. These groups represent phylogenetically robust clusters that are also phenotypically similar to each other. Similarities include observations *in vitro*, but more important are host associations. Isolates belonging to ssp. *fastidiosa* are primarily associated with grapevines, ssp. *multiplex* with almonds, oaks, and other trees, ssp. *pauca* with citrus and coffee, and ssp. *sandyi* with oleander.

Currently, the best method to determine the phylogenetic placement of novel isolates is MLST [21]. This method uses seven house-keeping loci spread through the genome of *X. fastidiosa*, which are all incorporated into the analysis. The main benefit of this approach is that all sequence data for type isolates is freely available at a database (http://pubmlst.org/xfastidiosa/), which currently contains 250 isolates (as of

June 2013). Most of the known *X. fastidiosa* genetic diversity is already represented in this database, so that phylogenetic coverage is adequate in most instances. Furthermore, there is no need to develop new typing tools, or request isolates or DNA for comparison purposes. In other words, once seven loci are sequenced (~500 bp each), one uses a database to infer its placement within the species. In addition to practical advantages, the use of a multi-locus approach is of relevance because *X. fastidiosa* is naturally competent for transformation [16], meaning that the sequence of a single gene does not necessarily reflect the phylogeny of the genome. Homologous recombination events have been detected between subspecies and may lead to incorrect inferences if loci used for tree-building have recombined [3, 20, 22].

The four subspecies likely evolved in geographic isolation, with ssp. *fastidiosa* having its center of origin in Central America, ssp. *multiplex* and ssp. *pauca* in North and South America respectively. Subspecies *sandyi* has only been found in the USA infecting oleander (*Nerium oleander*). However, evidence indicates recent anthropogenic movement of this bacterium, probably via contaminated plant material. Pierce's disease of grapevines in North America is caused by ssp. *fastidiosa* isolates, but those appear to have originated in Central America [19]. A very similar case has recently been reported from Taiwan [34], in which Pierce's disease isolates group within ssp. *fastidiosa*. Isolates of plum leaf scald in Brazil belong to ssp. *multiplex* [18]; because it is the only case of ssp. *multiplex* in South America, the assumption is that it was introduced. In the case of ssp. *pauca* and *multiplex*, there is evidence of between-subspecies recombination, raising the interesting possibility that horizontal gene transfer of novel alleles may lead to the emergence of new *X. fastidiosa* diseases [20].

The level of genetic diversity within subspecies is variable. For example, ssp. *sandyi* has little diversity and has only been found in oleander, while ssp. *multiplex* is subdivided into several clusters that colonize a wide range of tree species. The other two subspecies are also capable of causing disease in multiple host plant species, but in the case of ssp. *pauca* there are at least two genetic clusters, one which infects citrus and another coffee [3]. In general there is concordance between phylogenetic clustering and host plant association when MLST is used as a diagnostic tool.

## Diversity at the population level

The expectation that different genetic markers would be useful at different levels of resolution has also been supported for *X. fastidiosa* [3, 19]. DNA sequence-based

markers are useful at the species and subspecies levels, while fast-evolving markers such as tandem repeat regions are more useful at the population level. Although there is substantial genetic diversity within phylogenetic clusters of host-specific *X. fastidiosa*, few studies have addressed ecological questions other than estimating the degree of genetic diversity with unstructured populations. Therefore, little is known about *X. fastidiosa* populations.

A series of studies analyzed the spatial structure of citrus variegated chlorosis-causing *X. fastidiosa* in the state of São Paulo, Brazil. It was found that host plant variety did not affect genetic structure, and that populations were geographically isolated [5, 6]. In addition, a significant degree of clonality was observed in populations. Another study considered the structure of populations at a smaller spatial scale, by analyzing the ecology of *X. fastidiosa* isolates causing Pierce's disease of grapevines in Napa Valley, USA [7]. In this case, vineyards within ~10 km of each other were found to be infected with a highly diverse pathogen population. In addition to, essentially, a lack of clonality and high genetic diversity, isolates could not be genetically assigned to vineyards where they were collected, suggesting high rates of among-vineyard dispersal of genotypes.

## Horizontal gene transfer

Similarly to other bacteria, a range of processes leads to genetic diversity in *X. fastidiosa*. Horizontal gene transfer via transduction and transformation appears to be very important in *X. fastidiosa*. There is high diversity among prophage regions in *X. fastidiosa*, suggesting that bacteriophage infections are common in this pathogen [36], even though so far only one phage has been described for this bacterium [35]. MLST studies also suggested that homologous recombination was responsible for allele diversity, potentially at higher rates than point mutations [30]. Because homologous recombination rates have been reported to be larger than mutation rates for *X. fastidiosa in vitro* [16], it is possible recombination events are common in field populations. However, because recombination between identical sequences are undetectable, it is possible that its rate has been underestimated.

## SUMMARY

The bacterium *Xylella fastidiosa* is a genetically and phenotypically diverse plant pathogen. Although considered to have broad host range, it is becoming increasingly

evident that there is a high degree of phylogenetic cluster host specificity, and that *X. fastidiosa* infections of most plant species do not lead to disease symptoms. Therefore, it is important to understand the diversity of this pathogen, so that inferences of applied relevance such as host range and accurate detection protocols can be made in a robust manner. Recent advances in our knowledge about the genetic diversity of *X. fastidiosa* have not been followed by efforts to understand its phenotypically diversity, representing and important gap in information about the biology of this bacterium. Integrating all aspects of *X. fastidiosa* diversity is also relevant because it may provide insights into its evolutionary biology, and consequently processes leading to new and emerging diseases.

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